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CONTENTS:

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On the Anatomy of *Grindelia Squarrosa.*

BY NORA E. DALBEY.

Plates II-VII.

GRINDELIA SQUARROSA, popularly known as gum plant, is a widely distributed representative of the order Compositæ (4, 12, 13). In many respects it is a typical xerophyte. It is very hardy, with structural characteristics which enable it to withstand drouth, and with a habit of forming rosettes in the spring, which presumably helps it to withstand early frosts. With the exception of fungi, it has few natural enemies (8, 9).

A striking external characteristic is the presence of an aromatic gummy exudate over the entire surface. This exudate, which is secreted by glands, is excreted in great quantities from the flowers, while the glands on the leaf and stem always excrete an amount sufficient to impart a glutinous character very perceptible to the touch.

The plant presents some unusual structural features; and although it has been studied ecologically, but little anatomical work has been done upon it. Beauvais (1), in 1889, published an article on *Grindelia robusta*. His paper treats of the structrue as well as the chemistry of the plant. He calls the substance excreted from the epidermal glands a resin, similar to that found in the resin ducts in the interior collenchymatous layer.

Glasford (7), in 1898, published his work on the chemistry and histology of *Grindelia robusta*. He mentions resin glands

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on the stem, leaf, and involucre scales, also fragments of resin found in the flower. He characterizes the substance as very slightly soluble in water, readily soluble in benzene, alcohol, ether and chloroform, showing an acid reaction when in solution, and combined with the alkalies yielding an amorphous compound, which has the properties of resin soap.

THE LEAF.

The structure of the leaf was determined from specimens preserved in chloral hydrate, bleached in potassium hydroxide, and mounted in glycerine, and from material preserved in formalin, imbedded in paraffin, sectioned and stained with a double stain of safranin and Delafield's hæmatoxylin.

Anatomically the leaf presents some striking features. It averages 2.3 cm. in length, is rather thick and of a dull gray color, the dullness probably caused by crests and ridges of the cuticle.

When bleached it has the appearance of a pellucid network, due to the unusual character of the leaf tissues. (Figs. 1 and 3.) Water-storage tissue, made up of relatively large parenchyma cells, surrounds masses of other mesophyll cells that contain chloroplasts, but are not differentiated into palisade and spongy tissues. (Figs. 3, *m* and *h;* 5, *u* and *v;* and 6, *c* and *d.*) These chlorophyll-bearing cells resemble palisade tissue more than spongy tissue in both form and arrangement. Warming (18) mentions the fact that palisade tissue is greatly developed in xerophytes, either by an increase in the number of layers or in the height of the cells, and also mentions that there is a difference of opinion as to the significance and cause of this structural feature. He regards regulation of transpiration as the most essential reason for the structural differences, with light as a very important factor.

Clements (5) suggests that when the photosynthetic cells are all palisade cells, the condition may have been brought about by low water content.

The water-storage cells surround each vascular bundle, and extend to the epidermis above and below, thus giving the leaf the appearance of a network of large cells surrounding areas of smaller-celled tissue (fig. 5, *v* and *u*). A remarkable characteristic of the leaf is its capacity to hold water, for the water-storage cells occupy about one-third of the volume of the leaf (fig. 2, *e*).

There are a few thick, bud-shaped hairs on the margin of the leaf, which extend toward the apex, and project almost parallel with the margin of the leaf (figs. 13, *b*, and 14, *g*).

The chloroplast-bearing mesophyll cells are irregular in arrangement, some patches extending from surface to surface and others not (figs. 3 and 5). They vary from .006 mm. to .011 mm. in width, and from .016 mm. to .030 mm. in length. A single cell shows 8 to 16 chloroplasts of the usual type (fig. 17, *s*).

The leaf has a clasping base, and is entered by three vascular bundles. Near the base of the leaf, the tissue between these three bundles is composed of water-storage cells (figs. 1, *a*, and 7, *i*).

In cross section the vascular bundles are seen near the center of the leaf, midway between the upper and lower surfaces (figs. 3, *i*, and 5, *w*). The number in one cross section varies from 10 to 36.

The marginal veins unite in the serrations and form a coalescent mass of tracheids (figs. 1, 13, *c*, and 14, *i*). The tracheids have bordered pits, and spiral thickenings (fig. 20, *b* and *c*). The xylem and phloem are about equal in amount in the larger veins. They are separated by a layer of conjunctive parenchyma varying from one to three cells in thickness (fig. 4, *q*). The number of tracheids found in a cross section of a single vascular bundle varies from 5 to 37, and in the larger veins wood parenchyma and tracheal tissue alternate in radial rows, and bast fibers subtend the base of each bundle (fig. 4).

Resin ducts, on the average .028 mm. in diameter, are found near the phloem. They are surrounded by from 7 to 12 secreting cells (figs. 3, *l*, and 5, *y*).

The cells of the epidermis are rather thick-walled, and appear largest when seen from the surface (fig. 15, *k*). The outer walls are decidedly thickened, being on the average .007 mm. thick. A cuticle layer .0045 mm. thick is sculptured into irregular ridges, and in cross sections in places appears to be striated. In addition to this, cutinization goes deeper to the extent of .002 mm. in the walls of the epidermal cells which join the water-storage tissue.

A cuticle layer covers the walls of the guard cell where they bound the stomata, and extends back over the inner epidermal walls, thus lining the outer part of the stomatal

chamber (fig. 19, *a*). The epidermal cells are rich in protein, and in the young leaf they contain a small amount of starch.

The stomata are of the usual type (fig. 18). They are more numerous on the upper surface and over the areas of the chloroplast-bearing mesophyll (fig. 15), there being on the average 126 to the sq. mm. on the upper surface, and 97 to the sq. mm. on the lower surface. The guard cells are comparatively small, and are slightly raised above the surface of the epidermis (fig. 19). Such a position may be of physiological importance, since it increases the depth of the stomatal opening. Brown and Escombe (2) have shown by their experiments that whenever there is a constant difference maintained between the partial pressure of the vapor inside and outside the leaf, the amount of diffusion of water vapor is controlled by the depth of the apertures, and not by their areas, the rate of diffusion varying inversely with the depth of the stomatal opening. Thus the increased depth of the stomatal tube would decrease diffusion, which decrease may, however, be partly offset by the vapor density shells being in such a position that they are readily removed by wind.

Resin glands occur on the surface of the leaf, on the average 12 to the sq. mm. on the upper surface, and 3.4 to the sq. mm. on the lower surface. (Fig. 12.) They increase in number from the base of the leaf to its apex, and occur in clusters, often as many as thirty in a group at the apex, and near the tip of each tooth on the margin. (Fig. 14.) On the surface of the leaf the glands are usually sunken to a depth of about one-fourth the thickness of the leaf, and are always found in connection with the water-storage cells which surround the veins and extend to the epidermis. The layer of cells beneath the glands are usually elongated, and have comparatively thin walls (fig. 11, *u*) without a cuticle layer.

In cross section the glands are round, with an average diameter of .045, and are made up of from 20 to 60 cells. (Fig. 12, *a*.) A longitudinal section of a gland, however, presents three to four tiers of cells, with the outer layer very regular in form and arrangement. (Fig. 13, *b*.) The cells are rich in cell contents, and have prominent nuclei. On the surface of the leaf each gland has a thick coating of gum-resin, in the form of a minute droplet, while on the margin the entire cluster is covered by the same gummy excretion. On the upper surface, 9.4

per cent of the surface area is covered by these droplets, and on the lower surface 2.6 per cent. This gum-resin has a composite character. It swells and is slightly soluble in water, the solution showing an acid reaction. When a dry leaf was put into hot water and left for twenty-four hours the gum disappeared from the surface of the leaf, and that on the margin became viscous. Some of this gum-resin from the margin, after soaking in water twenty-four hours, was put in ether ten minutes, but did not dissolve. It is partially soluble in ether, alcohol and xylene, and when a small amount, placed on a slide, was irrigated with ether for a period of about ten minutes, a part dissolved, and the residue gave the reaction for gum when treated with methylene blue.

The substance shows the characteristic red stain when left twenty-four hours in a solution of alcanin, or in Sudan III, but it failed to show a resin reaction with copper acetate.

It may be possible that the tracheids at the margin of the leaf (fig. 13) function as water-storage cells, the resin serving to prevent transpiration in time of drouth; however, this is not a very satisfactory explanation of the relationship of the glands on the surface to the conducting and water-storage systems of the leaf. (Figs. 3, *k*, and 14.) It seems more probable that these glands function as organs of absorption. Kerner and Oliver (10) cite the case of an Aizoon having epidermal glands which serve to collect water and transfer it to the cells within the leaf. The gum resin, which swells in water, may be able to absorb the water from the rain and dew and transfer it, by osmosis, into the cells of the glands, whence it might pass into the noncutinized elongated cells of the epidermis and on into the adjoining water-storage tracheids (fig. 13), or into the water-storage cells of the mesophyll (figs. 3, *k*, and 11, *u*), and from these storage cells it could be slowly given off to the plant in time of drouth.

THE STEM.

The stem has a gray-green color, with a minutely roughish surface. In cross section it is round, with the outline broken by from three to eight protuberances, which occur at regular intervals. (Fig. 25.) Glands similar to those found on the leaf are distributed irregularly over the surface of the stem. The cross section shows a circle of open collateral bundles varying from ten to thirty-four in different sections. The stem is

mechanically strong, due to a large amount of well-developed supporting tissue, and an absence of large intercellular spaces. It contains a rather large proportion of conductive tissue. It is interesting to note here that in the publication by W. A. Cannon (2) on "Water Conducting Tissue of Some Desert Plants," he cites an experiment in which irrigated plants were poorer in conductive tissue than stems of the same diameter from non-irrigated plants.

Each stem bears a head of flowers, and a cross section at the base of the head shows a collenchyma zone fifteen to twenty cells deep. The collenchyma contains relatively large resin ducts .045 mm. in diameter. The resin glands are more numerous at the base of the flower than lower down on the stem.

The pith, comprising 20.3 per cent of the stem cross section, is composed of parenchymatous cells with comparatively thin and delicately pitted cellulose walls. (Fig. 23.) In cross section the pith cells vary in diameter from .015 to .067 mm. The walls of a layer of pith cells adjacent to the wood zone often become lignified. In the mature stem the pith cells are not empty, but contain some protein, a little starch, and numerous clusters of calcium oxalate crystals, made up of long, slender crystals, which give the cluster a spiny appearance. These crystals sometimes occur singly.

In the pith, and dispersed through the cortex and the wood parenchyma, there occur round to oval vesicles in which are minute globules that are stained a bright red when left for 24 hours in alcannin or in Sudan III. When sections were put into xylene and left 24 hours they did not show this stain reaction with alcannin and Sudan III. After being treated with xylene, the vesicles are present, and appear to be plastids which manufacture and store up oil.

The tracheal elements are in radial rows separated by relatively broad zones of wood fibers and parenchyma. (Fig. 22.) The large ducts occur in greater number near the inner part of the wood zone (fig. 26, *w*). In transverse section the vessels are round to oval in shape. The tracheal tubes and the tracheids have bordered pits, sometimes slightly elongated, but not enough to justify the use of the term scalariform (fig. 29, *g*), and single, double and triple spiral thickenings occur on the earlier vessels (fig. 29, *h*).

The ducts, the tracheids, the wood fibers, and the ray cells

of the xylem have lignified walls. One bundle of average size contained 176 vessels, of an average diameter of .11 mm. The vessels compose 3.74 per cent of the stem. The wood parenchyma is scanty in amount. It occurs in radial rows between the vessels and surrounds the inner margin of the bundles. (Fig. 26, *y*.) These cells contain granules which stain yellow with chlor-zinc-iodide. The wood fibers are of the characteristic type. (Fig. 26, *v*.) They average .0067 mm. in diameter, and are from .045 mm. to .150 mm. long. The walls, on the average .0013 mm. thick, are lignified and distinctly marked by slit-shaped straight pits.

The line of demarcation between the xylem and the phloem takes a sinuous course, as seen in fig. 25. In the stem near the base of the flower there is an equal amount of xylem and phloem, but in the portion lower down on the stem the ratio of xylem to phloem is 10 to 1. The phloem group is made up of small irregular cells with thin walls (fig. 30), and there is no differentiation into sieve tubes and companion cells. They gradually merge into the cambium layer. (Fig. 22, *i*.) In irregular masses the stain reaction with aniline sulphate and with safranin is characteristic of lignified walls. A longitudinal section shows lignification, extending in some cases the length of several cells and in other cases only part of the length of a single cell.

A complete cambium ring is not discernible.

The medullary rays in stem cross sections are narrow linear zones from two to six cells wide. Their distinguishing feature is a slight radial elongation of the cells. (Fig. 27, *b*.) The ray cells of the xylem are lignified, and at the sides they are bordered by a mass of xylem parenchyma cells resembling wood fibers in arrangement and in thickness of cell walls. (Fig. 27, *c*.) In longitudinal section these are brick-shaped, with simply pitted walls, and are usually empty of cell contents.

Within the pericycle exterior to each bundle is a mass of bast tissue. (Figs. 22, *n*, and 25, *s*.) Each bast group is radially from five to eight cells in thickness, and its cells are usually pentagonal in cross section. (Fig. 31.) The cell walls, on the average .004 mm. thick, are lignified, and are marked by straight pits. The average length of the bast fibers is about .11 mm., with long, tapering, interlacing ends.

The endodermis is very conspicuous. (Fig. 36, *p*.) In cross section the cells are round to oval and from .007 mm. to .045 mm. thick. In longitudinal section they are brick-shaped, on the average .088 mm. in length, and show a distinct nucleus. (Fig. 37, *s*.) These cells contain protein, and a small amount of starch. Sections stained twenty-four hours in alcannin and in Sudan III and washed with fifty per cent alcohol show all the walls heavily suberized with the exception of from one to three cells bordering the thin-walled parenchyma cells between the bast groups. (Fig. 36.) These cells have comparatively thin cellulose walls, and they are clearly places of radial conduction.

The central band of the cortex is composed of collenchyma cells with an average diameter of .022 mm. and an average length of .120 mm. (Figs. 25, *q*, and 34, *j*.) They have little uniformity in size and shape, with the exception of one layer which lies adjacent to the epidermis and resembles the epidermis in structure and in cell contents. (Fig. 34.) The cellulose walls contain straight pits. Stomata occur on the stem in connection with rather large aërating spaces. Resin ducts occur adjacent to the bast. They are in cross section oval to round in shape, .015 mm. to .045 mm. in diameter, and are surrounded by from seven to twelve secreting cells rich in cell contents.

The epidermis consists of one layer of cells, which have thick cellulose walls. The outer wall is .007 mm. thick and the other walls each .0015 mm. thick. The lumen of the cells is nearly round, and has an average diameter of .011 mm. The epidermal cells contain chloroplasts of the usual type, and granules which show the characteristic stain reaction for protein. There is a very marked cuticle .0045 mm. thick, with deep ridging, which often accompanies great thickness. Stomata the same in structure as the stomata of the leaf are scattered over the surface, on the average nine to the sq. mm.

The inflorescence is a head surrounded by an involucre of spirally arranged bracts. Before the bud opens a large drop of gum-resin is excreted, which covers the buds with a waxy coating. Small disk flowers occur in the center of the head, surrounded by a circle of golden-yellow ray flowers. A transverse section through the top of one of the involucre bracts is almost round, with a diameter of .52 mm.

Toward the base of the bract the outline approaches triangular, being flattened on one side. Vascular bundles consisting of a large amount of phloem (fig. 35, *l*) occur near the central part of the bract, and in cross section are arranged in the shape of an arch. (Fig. 35.) The upper part of the bract contains large masses of mesophyll cells separated by water-storage tissue, while the lower part is made up of more scattered masses of mesophyll and a larger amount of water-storage parenchyma tissue, which often contains large aërating spaces. Resin ducts are found in the parenchyma adjacent to each bundle, the central one reaching a diameter of .03 mm. The mesophyll cells appear to contain more chloroplasts than the mesophyll of the leaf.

The epidermis of the bract is the same in structure as the epidermis of the leaf, and contains stomata and a large number of resin glands similar to those on the leaf. There is a thick cuticle layer covering the epidermis.

SUMMARY.

1. The stem, leaves and involucre scales are covered with an aromatic sticky exudate.

2. This exudate comes from multicellular superficial glands that occur as frequently as 1.2 to the sq. mm. on the upper surface and 3.4 to the sq. mm. on the lower surface of the leaf.

3. The exudate appears to be essentially a gum-resin, some of its substance being soluble in water, some in ether, alcohol, and in xylene, and it stains as mucilage does with methylene blue, and as oils and resins do with alcannin and Sudan III.

4. Where the glands occur at the tips of the marginal serrations clusters of tracheids extend to them from the marginal veins, and where they occur over the surface of the leaf they are subtended by masses of water-storage cells.

5. These water-storage cells compose about one-third of the mesophyll; they surround the veins, and often extend from one surface to the other.

6. It would seem from their position and the nature of their exudate that the glands might serve in the absorption of water from dew or rain. The gum of the exudate could imbibe the water and give it over to the storage tracheids and parenchyma, and the resin could protect against evaporation.

7. Resin ducts occur adjacent to the veins of stem, leaves and flowers.

8. The leaf is entered by three vascular bundles, and near the base the tissue between the veins is composed of water-storage parenchyma.

9. The chloroplast-bearing cells of the mesophyll are not differentiated into palisade and spongy parenchyma.

10. In the phloem of the stem there is no differentiation into sieve tubes and companion cells, and irregular patches of thin-walled phloem cells show more or less lignification of the walls.

11. In the stem the cell walls of the conspicuous endodermis are suberized, excepting from one to three cells that border the thin-walled parenchyma of the pericycle standing between the bundles of bast fibers. These uncutinized passage cells of the endodermis are undoubtedly places where translocation of materials takes place between the outer and inner bark.

12. The stem is made strong by a large development of wood, bast and collenchyma.

The material selected for this investigation was obtained from Scott county, Kansas, in midsummer, at the height of the flowering season. The study of the anatomy was carried on in the research laboratory of the botanical department of the University of Kansas, under the direction of Prof. W. C. Stevens, to whom his student gratefully acknowledges her appreciation of his help and interest. Thanks are due to Mr. L. M. Peace, preparator and demonstrator in the botanical laboratory, for his invaluable suggestions and assistance during the progress of the investigation.

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